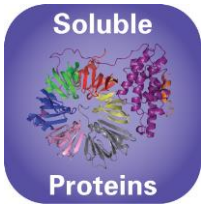
Molecular Dimensions



The BCS Eco Screen MD1-104-ECO

A 96 condition protein crystallization screen incorporating PEG Smears[1] for broader chemical space coverage.

MD1-104-ECO[2] is presented as 96 x 10 mL conditions.

Features of The BCS Eco Screen:

- Contains defined PEG Smears – efficient screening of chemical space.
- Wide PEG coverage.
- Screen against multiple additives. Contains multiple salts/additives shown to promote crystallization.
- Proven crystallization successes with multiple proteins.
- Cacodylate-free version.

Introduction

The Basic **C**hemical **S**pace (**BCS) Screen** developed at the Structural Genomics Consortium (SGC), Oxford, UK, has been formulated to include PEG smears - enabling a broader search of chemical space than just using individual PEGS.

The benefit of using PEG smears is that it reduces the number of PEG variables while maintaining a large coverage of PEG space. The PEGs in **The BCS Screen** are grouped by molecular weight and mixed to create four PEG smears (available to purchase separately). The smears cover a broader range of chemical space while reducing the number of PEG variables.

The BCS Screen was tested for crystallization potency using a set of 191 proteins from projects at the Structural Genomics Consortium (SGC) based in Oxford, UK. The success rates of the smear-based screen were similar to the combined success rates of the other four in-house screens exploited regularly at the SGC. In particular, the ability of the smear-based screen promoted crystal growth of several proteins that had been difficult to crystallize (Figure 1) using the commercial sets.

The BCS Screen: The first 24 conditions in The BCS Screen form a grid screen of the four PEG smears against pH (range 4.5-9.5). The remaining 72 conditions consist of a sparse matrix screen in which the precipitant is always one of the PEG smears but with a variety of additives and buffers. The additives include some of the more unusual crystal-promoting agents such as Rubidium chloride (also useful for phasing) and ammonium nitrate. More common additives including Sodium Bromide (useful for phasing) and glycerol are also used in the screen. In addition, multiple salts/additives are implemented, which have been shown also to promote crystallization.

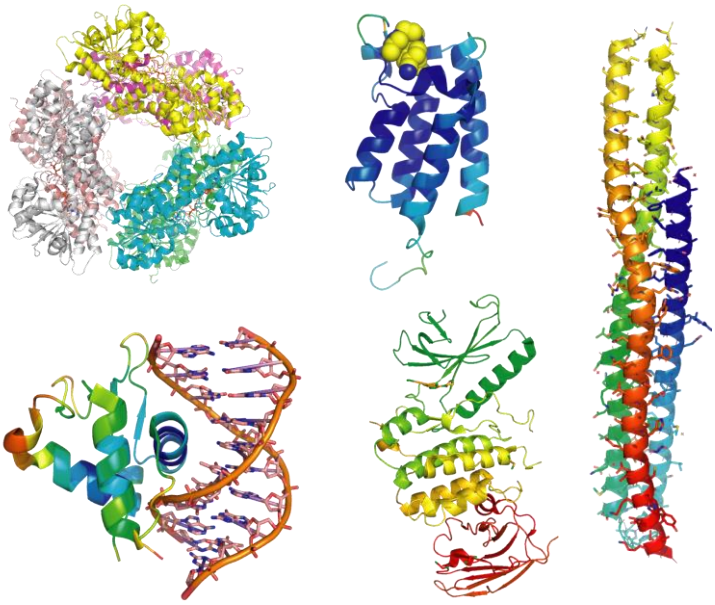


*Figure 1. Examples of crystal structures obtained from using **The BCS Screen** at the SGC. For example, single-domain proteins, protein-protein complex and protein-DNA complex.*

The BCS Screen is formulated in collaboration with, Apirat Chaikuad, Elizabeth MacLean, Stefan Knapp and Frank von Delft at the Structural Genomics Consortium, Oxford, UK.

[1] PEG smears, originally suggested by Janet Newman (ActaD 2005)

[2] Sodium cacodylate is replaced with MES

PEG Smear compositions used in The BCS Screen

Eleven PEGs were selected from those commonly used in most commercial screens (Figure 2) to provide molecular weight (MW) coverage from 400 to 10k Da, namely PEG 400, 550MME, 600, 1K, 2K, 3350, 4K, 5KMME, 6K, 8K and 10K.
These eleven PEGs were then divided into three classes based on molecular weight, low (≤1k Da), medium (>1k-5k Da), and high (≥6k Da).

The four smears in The BCS Screen consist of:

PEG Smear	Composition	Catalogue Number (100 mL)	Catalogue Number (250 mL)
50% v/v PEG Smear Low	12.5%v/v PEG 400 12.5%v/v PEG 500 MME 12.5%v/v PEG 600 12.5%w/v PEG 1000	MD2-100-258	MD2-250-258
50% v/v PEG Smear Medium	12.5%w/v PEG 3350 12.5%w/v PEG 4000 12.5%w/v PEG 2000 12.5%w/v PEG 5000 MME	MD2-100-259	MD2-250-259
50% v/v PEG Smear High	16.67%w/v PEG 8000 16.67%w/v PEG 10000 16.67%w/v PEG 6000	MD2-100-260	MD2-250-260
50% v/v PEG Smear Broad*	4.55%v/v PEG 400 4.55%v/v PEG 500 MME 4.55%v/v PEG 600 4.55%w/v PEG 1000 4.55%w/v PEG 2000 4.55%w/v PEG 3350 4.55%w/v PEG 4000 4.55%w/v PEG 5000 MME 4.55%w/v PEG 6000 4.55%w/v PEG 8000 4.55%w/v PEG 10000	MD2-100-261	MD2-250-261

***** The Broad Smear is similar to the originally described smear (Newman et al., 2005), which did not however contain any methylated polymers.

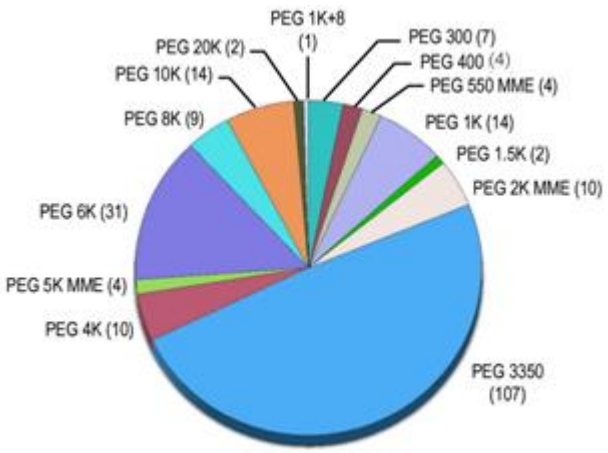


Figure 2. Bias in PEG molecular weights in four of the most common sparse matrix screens. Taken from Chaikuad *et al* (2015).

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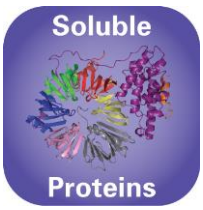


Formulation Notes:

The BCS Eco Screen reagents are formulated using ultrapure water (>18.0 MΩ) and are sterile-filtered using 0.22 µm filters. No preservatives are added.

Final pH may vary from that specified on the datasheet. Molecular Dimensions will be happy to discuss the precise formulation of individual reagents.
Individual reagents and stock solutions for optimization are available from Molecular Dimensions.

Enquiries regarding **The BCS Eco Screen** formulation, interpretation of results or optimization strategies are welcome. Please e-mail, fax or phone your query to Molecular Dimensions.

Contact and product details can be found at www.moleculardimensions.com

Hints & Tips

The BCS Eco Screen conditions are not cryoprotected, so cryoprotection will have to be carried out. For this we recommend CryoProtX (MD1-61).

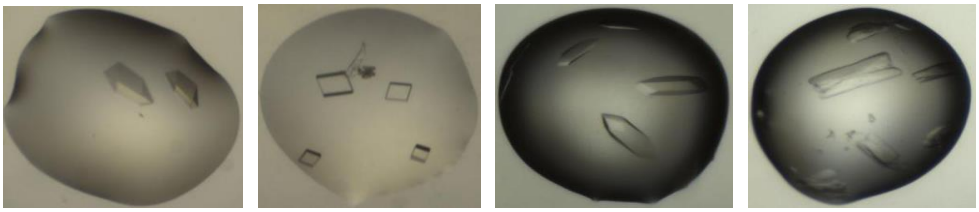
To Optimize PEG Smears we recommend the following: treat the PEG Smears as 'normal PEG' and just vary the concentration, or deconvolute the effects of the individual PEGs as described in Chaikuad *et al* (2015).

The screen has been predominantly tested with soluble proteins, but this does not mean it cannot work for membrane proteins.

Publications resulting from crystals grown using The BCS screen include:

Chaikuad, A., Knapp, S. & von Delft, F. (2015). Acta Cryst. D71, 1627-1639.
Froese DS *et al*, (2010) J Biol Chem. Dec 3;285(49):38204-13.
Filippakopoulos P *et al*, (2012) Cell. Mar 30;149(1):214-31.
Chaikuad A *et al*, (2011) Proc Natl Acad Sci U S A, Dec 27; 108(52):21028-33.
Chaikuad A *et al*, (2014) Nat Chem Biol. Oct;10(10):853-60.
Kovackova S *et al*,(2015) J Med Chem. Apr 23;58(8):3393-410
Egger S *et al*, (2012) J Biol Chem. Jan 13;287(3):2119-29
Chaikuad A *et al*, (2016) J Med Chem. Feb 25;59(4):1648-53
Chen P *et al*, (2016) J Med Chem. Feb 25;59(4):1410-24
Drouin L *et al*, (2015) J Med Chem. Mar 12;58(5):2553-9

*Protein crystals grown using **The BCS Screen**. Images courtesy of the SGC, Oxford.*



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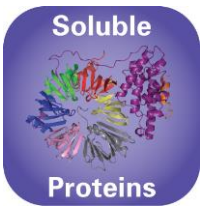
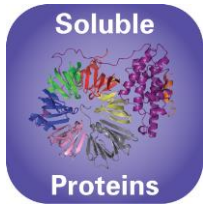


Re-Ordering details:

Catalogue Description	Pack size	Catalogue Code
The BCS Screen	96 x 10 mL	MD1-104
The BCS Eco Screen	96 x 10 mL	MD1-104-ECO
The BCS Screen HT-96	96 x 1 mL	MD1-105
The BCS Screen HT-96 Eco Screen	96 x 1 mL	MD1-105-ECO
The BCS Screen FX-96	96 x 100 µL	MD1-105-FX
Single Reagents		
The BCS Screen	100 mL	MDSR-104-tube number
The BCS Screen HT-96	100 mL	MDSR-105-well number
50% v/v PEG Smear Low	100/250 mL	MD1-100(250)-258
50% v/v PEG Smear Medium	100/250 mL	MD1-100(250)-259
50% v/v PEG Smear High	100/250 mL	MD1-100(250)-260
50% v/v PEG Smear Broad	100/250 mL	MD1-100(250)-261

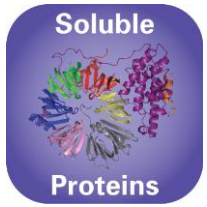
Molecular Dimensions



The BCS Eco Screen Conditions 1-48 (Box 1) MD1-104-ECO

Tube #	Conc.	Salt1	Conc.	Salt2	Conc.	Buffer	pH	Conc.	Precipitant1	Conc.	Precipitant2
1-1					0.1 M	Sodium acetate	4.5	30 % v/v	PEG Smear Low		
1-2					0.1 M	Phosphate/Citrate	5.5	30 % v/v	PEG Smear Low		
1-3					0.1 M	MES	6.5	30 % v/v	PEG Smear Low		
1-4					0.1 M	Sodium acetate	4.5	25 % v/v	PEG Smear Medium		
1-5					0.1 M	Phosphate/Citrate	5.5	25 % v/v	PEG Smear Medium		
1-6					0.1 M	MES	6.5	25 % v/v	PEG Smear Medium		
1-7					0.1 M	Sodium acetate	4.5	20 % v/v	PEG Smear High		
1-8					0.1 M	Phosphate/Citrate	5.5	20 % v/v	PEG Smear High		
1-9					0.1 M	MES	6.5	20 % v/v	PEG Smear High		
1-10					0.1 M	Sodium acetate	4.5	22 % v/v	PEG Smear Broad		
1-11					0.1 M	Phosphate/Citrate	5.5	22 % v/v	PEG Smear Broad		
1-12					0.1 M	MES	6.5	22 % v/v	PEG Smear Broad		
1-13					0.1 M	HEPES	7.5	30 % v/v	PEG Smear Low		
1-14					0.1 M	Tris	8.5	30 % v/v	PEG Smear Low		
1-15					0.1 M	BICINE	9.3	30 % v/v	PEG Smear Low		
1-16					0.1 M	HEPES	7.5	25 % v/v	PEG Smear Medium		
1-17					0.1 M	Tris	8.5	25 % v/v	PEG Smear Medium		
1-18					0.1 M	BICINE	9.3	25 % v/v	PEG Smear Medium		
1-19					0.1 M	HEPES	7.5	20 % v/v	PEG Smear High		
1-20					0.1 M	Tris	8.5	20 % v/v	PEG Smear High		
1-21					0.1 M	BICINE	9.3	20 % v/v	PEG Smear High		
1-22					0.1 M	HEPES	7.5	22 % v/v	PEG Smear Broad		
1-23					0.1 M	Tris	8.5	22 % v/v	PEG Smear Broad		
1-24					0.1 M	BICINE	9.3	22 % v/v	PEG Smear Broad		
1-25								35 % v/v	PEG Smear Low		
1-26	0.2 M	Ammonium acetate			0.1 M	Sodium acetate	4.6	28 % v/v	PEG Smear Low	5 % v/v	Ethylene glycol
1-27	0.15 M	Sodium chloride						28 % v/v	PEG Smear Medium		
1-28	0.2 M	Ammonium sulfate			0.1 M	MES	5.5	25 % v/v	PEG Smear Medium		
1-29	0.1 M	Sodium/potassium phosphate pH 5.5	0.1 M	Rubidium chloride	0.1 M	Sodium citrate	5.5	25 % v/v	PEG Smear Medium		
1-30	0.2 M	Potassium chloride						22.5 % v/v	PEG Smear High		
1-31	0.15 M	Ammonium acetate			0.1 M	Sodium citrate	5.0	15 % v/v	PEG Smear High		
1-32	0.05 M	L-Arginine	0.05 M	L-Glutamic acid monosodium salt hydrate				28 % v/v	PEG Smear Broad	5 % v/v	Glycerol
1-33	0.15 M	Magnesium acetate tetrahydrate			0.1 M	Sodium citrate	5.6	20 % v/v	PEG Smear Broad		
1-34	0.2 M	Ammonium sulfate			0.1 M	Sodium acetate	4.6	25 % v/v	PEG Smear Broad		
1-35	0.2 M	Potassium sodium tartrate tetrahydrate			0.1 M	MES	6.0	25 % v/v	PEG Smear Low		
1-36	0.1 M	Calcium chloride dihydrate	0.1 M	Magnesium chloride hexahydrate	0.1 M	PIPES	7.0	22.5 % v/v	PEG Smear Medium		
1-37	0.2 M	Ammonium nitrate			0.1 M	MES	5.3	22.5 % v/v	PEG Smear Low		
1-38					0.1 M	MES	6.5	22.5 % v/v	PEG Smear Low	10 % v/v	2-Propanol
1-39	0.15 M	Ammonium nitrate			0.1 M	MES	6.0	20 % v/v	PEG Smear Medium	5 % v/v	Ethylene glycol
1-40	0.2 M	Sodium formate			0.1 M	Sodium phosphate	6.2	20 % v/v	PEG Smear Medium	10 % v/v	Glycerol
1-41	0.2 M	Lithium sulfate			0.1 M	ADA	6.5	30 % v/v	PEG Smear Medium		
1-42	0.1 M	Potassium thiocyanate	0.1 M	Sodium bromide	0.1 M	MES	6.5	12 % v/v	PEG Smear High		
1-43	0.2 M	Ammonium sulfate			0.1 M	ADA	6.5	18 % v/v	PEG Smear High		
1-44	0.15 M	Calcium chloride dihydrate			0.1 M	MES	6.2	15 % v/v	PEG Smear Broad	5 % v/v	Glycerol
1-45	5 % v/v	T-mate pH 7.0			0.1 M	MES	5.3	15 % v/v	PEG Smear Broad	10 % v/v	Ethylene glycol
1-46	0.2 M	Sodium chloride			0.1 M	Sodium phosphate	6.2	28 % v/v	PEG Smear Broad		
1-47	0.1 M	Ammonium sulfate	0.05 M	Magnesium sulfate heptahydrate	0.1 M	Sodium citrate	5.5	22.5 % v/v	PEG Smear Medium		
1-48	0.01 M	Cobalt(II) chloride hexahydrate	0.2 M	Magnesium chloride hexahydrate	0.1 M	Bis-Tris propane	8.0	22.5 % v/v	PEG Smear Medium	2 % v/v	Glycerol

Molecular Dimensions



The BCS Eco Screen Conditions 1-48 (Box 2) MD1-104-ECO

Tube #	Conc.	Salt1	Conc.	Salt2	Conc.	Buffer	pH	Conc.	Precipitant1	Conc.	Precipitant2
2-1	0.08 M	Magnesium acetate tetrahydrate	0.02 M	Magnesium chloride hexahydrate	0.1 M	MES	6.5	25 % v/v	PEG Smear Low		
2-2	0.1 M	Potassium chloride			0.1 M	HEPES	7.5	16 % v/v	PEG Smear Low	5 % v/v	Ethylene glycol
2-3	0.1 M	Zinc acetate dihydrate	0.1 M	Zinc chloride	0.1 M	Bis-tris	7.5	20 % v/v	PEG Smear Medium		
2-4	0.1 M	Magnesium chloride hexahydrate	0.1 M	Potassium chloride	0.1 M	PIPES	7.0	20 % v/v	PEG Smear Medium		
2-5	0.05 M	Magnesium sulfate heptahydrate			0.1 M	HEPES	7.5	28 % v/v	PEG Smear Medium		
2-6	0.1 M	Sodium/potassium phosphate pH 7.5			0.1 M	HEPES	7.5	15 % v/v	PEG Smear High	10 % v/v	Ethylene glycol
2-7	0.1 M	Magnesium formate dihydrate	0.1 M	Rubidium chloride	0.1 M	PIPES	7.0	25 % v/v	PEG Smear High		
2-8	0.2 M	Lithium sulfate			0.1 M	HEPES	7.2	25 % v/v	PEG Smear Broad		
2-9	0.2 M	Ammonium nitrate			0.1 M	HEPES	7.5	20 % v/v	PEG Smear Broad		
2-10	0.1 M	Magnesium chloride hexahydrate	0.1 M	Rubidium chloride	0.1 M	HEPES	7.5	30 % v/v	PEG Smear Broad		
2-11	0.05 M	Magnesium chloride hexahydrate	0.05 M	Sodium citrate tribasic dihydrate	0.1 M	Bis-Tris propane	7.8	22.5 % v/v	PEG Smear High		
2-12	7 % v/v	T-mate pH 7.0			0.1 M	BICINE	9.0	22.5 % v/v	PEG Smear High	10 % v/v	Ethylene glycol
2-13	0.15 M	Sodium citrate tribasic dihydrate			0.1 M	HEPES	7.8	25 % v/v	PEG Smear Low		
2-14	0.2 M	Sodium chloride			0.1 M	Tris	8.5	28 % v/v	PEG Smear Low	5 % v/v	Glycerol
2-15	0.075 M	Sodium acetate trihydrate	0.15 M	Sodium chloride	0.1 M	Tris	8.0	15 % v/v	PEG Smear Medium		
2-16	0.1 M	Sodium chloride	0.1 M	Sodium formate	0.1 M	Bis-Tris propane	8.5	25 % v/v	PEG Smear Medium		
2-17	0.2 M	Ammonium sulfate	0.05 M	Magnesium sulfate heptahydrate	0.1 M	BICINE	9.0	20 % v/v	PEG Smear Medium		
2-18	0.2 M	Ammonium nitrate			0.1 M	Bis-Tris propane	8.5	18 % v/v	PEG Smear High		
2-19	0.2 M	Magnesium chloride hexahydrate			0.1 M	Tris	8.0	25 % v/v	PEG Smear High	10 % v/v	Glycerol
2-20	0.15 M	Ammonium acetate	0.01 M	Calcium chloride dihydrate	0.1 M	Tris	8.5	28 % v/v	PEG Smear Broad		
2-21					0.1 M	BICINE	9.0	25 % v/v	PEG Smear Broad	10 % v/v	2-Propanol
2-22	0.2 M	Ammonium sulfate			0.1 M	Tris	8.0	20 % v/v	PEG Smear Broad		
2-23	0.02 M	Magnesium sulfate heptahydrate	0.2 M	Potassium chloride	0.1 M	BICINE	8.8	22.5 % v/v	PEG Smear Broad		
2-24	0.1 M	Potassium sodium tartrate tetrahydrate			0.1 M	MES	5.5	22.5 % v/v	PEG Smear Broad	10 % v/v	Ethylene glycol
2-25	0.01 M	Cobalt(II) chloride hexahydrate	0.1 M	Magnesium formate dihydrate	0.1 M	MES	6.2	14 % v/v	PEG Smear Low		
2-26	0.15 M	Lithium sulfate	0.05 M	Magnesium chloride hexahydrate	0.1 M	Bis-Tris	6.8	25 % v/v	PEG Smear Low		
2-27	0.2 M	Ammonium sulfate	0.01 M	Cadmium chloride hemi(pentahydrate)	0.1 M	HEPES	7.5	25 % v/v	PEG Smear Medium		
2-28	0.1 M	Potassium chloride	0.1 M	Magnesium chloride hexahydrate				18 % v/v	PEG Smear Medium	10 % v/v	Ethylene glycol
2-29	0.1 M	Magnesium acetate tetrahydrate			0.1 M	MES	6.5	12 % v/v	PEG Smear Medium	10 % v/v	Ethylene glycol
2-30	0.1 M	Magnesium acetate tetrahydrate	0.1 M	Potassium chloride	0.1 M	MES	6.2	12 % v/v	PEG Smear High		
2-31	0.04 M	Calcium chloride dihydrate	0.04 M	Sodium formate	0.1 M	PIPES	7.0	8 % v/v	PEG Smear High		
2-32	0.075 M	Magnesium chloride hexahydrate	0.075 M	Sodium citrate tribasic dihydrate	0.1 M	Bis-Tris	6.0	18 % v/v	PEG Smear Broad		
2-33	0.1 M	Magnesium chloride hexahydrate	0.1 M	Sodium acetate trihydrate	0.1 M	Bis-Tris	6.5	15 % v/v	PEG Smear Broad		
2-34	0.1 M	Ammonium sulfate	0.1 M	Sodium formate	0.1 M	HEPES	7.0	25 % v/v	PEG Smear Broad		
2-35	0.2 M	Sodium/potassium phosphate pH 7.5			0.1 M	HEPES	7.5	22.5 % v/v	PEG Smear Medium	10 % v/v	Glycerol
2-36	0.3 M	Sodium chloride	0.05 M	L-Arginine	0.1 M	Tris	7.5	22.5 % v/v	PEG Smear Broad	0.05 M	L-Glutamic acid monosodium salt hydrate
2-37	0.04 M	Calcium chloride dihydrate	0.04 M	Sodium formate	0.1 M	Tris	8.0	25 % v/v	PEG Smear Low		
2-38	0.1 M	Magnesium chloride hexahydrate	0.1 M	Rubidium chloride	0.1 M	PIPES	7.0	20 % v/v	PEG Smear Low		
2-39	0.2 M	Magnesium chloride hexahydrate	10 % v/v	Ethylene glycol	0.1 M	HEPES	7.5	15 % v/v	PEG Smear Medium	5 % v/v	2-Propanol
2-40	0.05 M	Ammonium acetate	0.15 M	Magnesium sulfate heptahydrate	0.1 M	HEPES	7.0	12 % v/v	PEG Smear Medium		
2-41	7 % v/v	T-mate pH 7.0			0.1 M	HEPES	7.2	20 % v/v	PEG Smear Medium		
2-42	0.1 M	Ammonium acetate	0.1 M	Zinc chloride	0.1 M	Bis-Tris	7.2	15 % v/v	PEG Smear High		
2-43	0.15 M	Lithium sulfate	0.05 M	Magnesium chloride hexahydrate	0.1 M	HEPES	7.8	20 % v/v	PEG Smear High		
2-44	0.1 M	Potassium thiocyanate	0.1 M	Sodium bromide	0.1 M	Tris	7.8	25 % v/v	PEG Smear Broad		
2-45	0.05 M	Ammonium sulfate	0.05 M	Lithium sulfate	0.1 M	Bis-Tris propane	8.5	28 % v/v	PEG Smear Broad		
2-46	0.2 M	Ammonium sulfate	0.01 M	Cadmium chloride hemi(pentahydrate)	0.1 M	PIPES	7.0	15 % v/v	PEG Smear Broad	10 % v/v	Ethylene glycol
2-47	0.2 M	Lithium sulfate	0.05 M	Zinc acetate dihydrate	0.1 M	Bis-Tris	7.5	22.5 % v/v	PEG Smear Broad		
2-48	0.075 M	Sodium bromide	0.05 M	Sodium fluoride	0.1 M	HEPES	7.8	22.5 % v/v	PEG Smear Broad	0.075 M	Sodium iodide